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INTERACTION OF A MAMMALIAN SINGLE STRAND SPECIFIC DNA BINDING PROTEIN WITH DNA POLYMERASE α

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1. Introduction

Single strand specific DNA binding proteins from mammalian cells can stimulate the activity of DNA polymerase α but not of DNA polymerase β from the same cells and not the activities of bacterial DNA polymerases [1,2]. We now report more detailed studies about the mechanism of this stimulation process and show that the binding protein increases the affinity of DNA polymerase α to its primer template.

2. Materials and methods

2.1. *Cultivation of bovine lymphocyte and mouse ascites cells*

This was as in [1,3]. Standard buffer contains 50 mM Tris–HCl, pH 7.5; 1 mM EDTA; 10 mM 2-mercaptoethanol and 20% (v/v) glycerol.

2.2. *Preparation of DNA polymerase α and DNA binding protein*

Both proteins from mouse ascites cells as well as from growing bovine lymphocytes were prepared as in [1,3]. The DNA polymerase α fraction (purification ~ 2000-fold) was completely free of nuclease activity. The binding protein was further purified after the last column step by heating at 60°C for 15 min in the presence of bovine serum albumin (0.5 mg/ml). Precipitated proteins were removed by centrifugation at 10 000 × *g* and 0°C for 10 min. The binding protein was recovered in the supernatant. No loss of binding and stimulating activity was observed [4]. The heat step eliminates all contaminating nucleolytic activity.

2.3. *Assays*

Assays of DNA binding protein [1], endo- and exonuclease activities [5] were carried out as described. DNA polymerase α activity was assayed as described [1] if not stated otherwise in the legends.

2.4. *Other techniques*

Preparation of single strand DNA cellulose [6], 'activation' of calf thymus DNA [7] and determination of protein concentration [8] were done as described.

3. Results

Experiments are presented in fig.1 which demonstrate that binding protein increases the fraction of DNA bound α-polymerase at salt concentrations of 20–40 mM.

Fig.2 shows the stimulation of DNA polymerase α activity by binding protein at increasing salt concentrations. Single stranded DNA is used as primer template [1]. Two effects are observed: First, the binding protein stimulates the DNA polymerase α activity at all salt concentrations used and, second, the optimal salt concentration in the presence of the binding protein is shifted to 30–40 mM NaCl, a concentration which inhibits the activity of the DNA polymerase α in the absence of binding protein.

The data of fig.2 suggest that the stimulation of

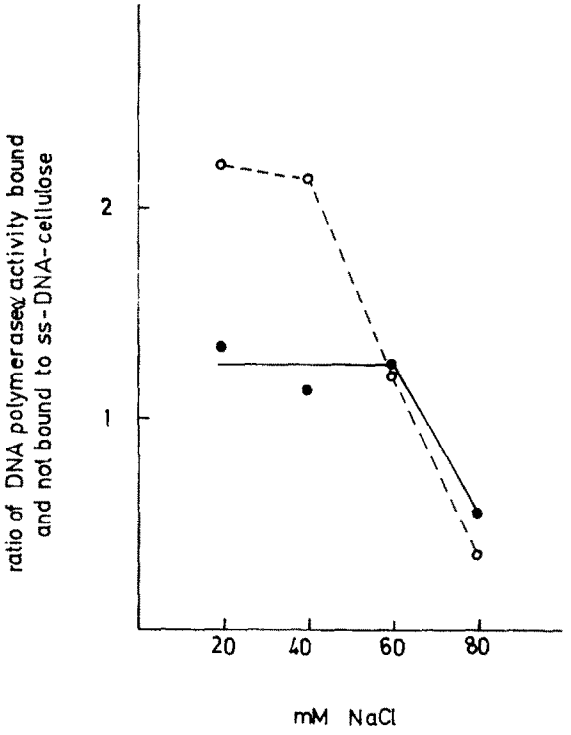

Fig.1. The association of DNA polymerase α with single stranded DNA at different NaCl concentrations in the presence of binding protein. Eight samples of 0.4 ml DNA cellulose in standard buffer complemented with 1 mg/ml bovine serum albumin were prepared. DNA polymerase α, 20 units, were added to each sample. Four samples received in addition 150 μg binding protein. The volume of all samples were then adjusted to 0.8 ml by addition of standard buffer. Four sets of two samples each, one containing α-polymerase plus binding protein and one containing only α-polymerase, were then dialysed for 2 h at 4°C against standard buffer with 20 mM, 40 mM, 60 mM and 80 mM NaCl, respectively. After dialysis, non-adsorbed α-polymerase was recovered in the supernatants of a 15 000 × *g* centrifugation at 4°C for 20 min (supernatant 1). DNA-bound α-polymerase was eluted by resuspending the first DNA cellulose pellet in 0.4 ml standard buffer containing 250 mM NaCl. After stirring for 30 min, the DNA cellulose was pelleted again. The supernatant 2 contained all DNA-bound α-polymerase (whereas all added DNA binding protein was released by a salt concentration of 1.0 M NaCl). DNA polymerase α activity was assayed in all fractions with activated calf thymus DNA as in section 2. Total recovery of α-polymerase was about the same for all samples. The ratio of recovered α-polymerase activity in supernatant 2 and recovered activity in supernatant 1 was taken as a measure for the binding of α-polymerase to DNA. These ratios are plotted above as a function of initial salt concentrations.

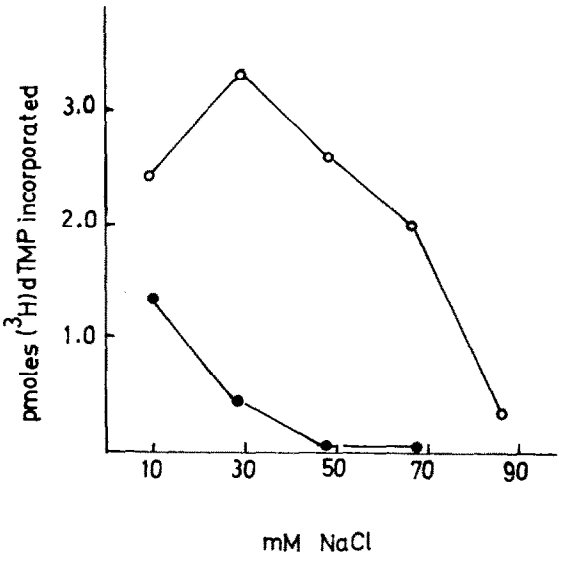

Fig.2. Optimal NaCl concentration for α-polymerase in the absence of binding protein. Heat denatured calf thymus DNA, 10 μg in 0.05 ml vols. standard buffer were incubated with 25 μg binding protein (from ascites cells). In control samples, the protein was omitted. After preincubation for 5 min at 37°C, 0.1 ml vol. polymerase reaction mixture containing, 20 mM, 40 mM, 60 mM and 80 mM NaCl, respectively, were added. Polymerisation was started after the addition of 0.25 units DNA polymerase α, and incorporation of radioactive material was determined after an incubation of 1 h at 37°C. (•) d[^{3}H]TMP incorporated in the absence of DNA-binding protein; (○) d[^{3}H]TMP incorporated in the presence of DNA-binding protein.

the polymerisation reaction may be due to an increased affinity of DNA polymerase α to its primer template in the presence of the binding protein.

In the absence of binding protein the DNA polymerase α leaves the primer template after a few polymerisation steps and then reassociates again (distributive synthesis [9]). The α-polymerase activity therefore strongly depends on the concentration of the enzyme itself and the primer template. A dilution of both components should decrease the polymerizing activity.

Figure 3 shows as expected that, using single stranded DNA as primer template, the DNA polymerase α activity becomes completely inhibited by a 3- and 10-fold dilution. In the presence of the binding protein, however, the rate of DNA synthesis

does not change very much after dilution. Control experiments (data not shown) exclude the possibility that the DNA polymerase α becomes inactivated by such a dilution.

4. Discussion

We have performed the experiments reported in this communication to provide for a better understanding of binding protein–α-polymerase interaction. Our earlier experiments [1] had shown that this interaction is specific since the reaction catalysed by DNA polymerase α is stimulated by binding protein but not that catalysed by the eukaryotic β polymerase and by microbial DNA polymerases. This suggested an association of binding protein and α polymerase. We therefore have tried to detect the formation of a complex between both components as has been shown by others [10] to occur between bacteriophage T4 DNA polymerase and T4-gene 32 binding protein. We have investigated a large variety of conditions but we could never observe a binding protein–polymerase complex by sucrose gradient analysis (unpublished).

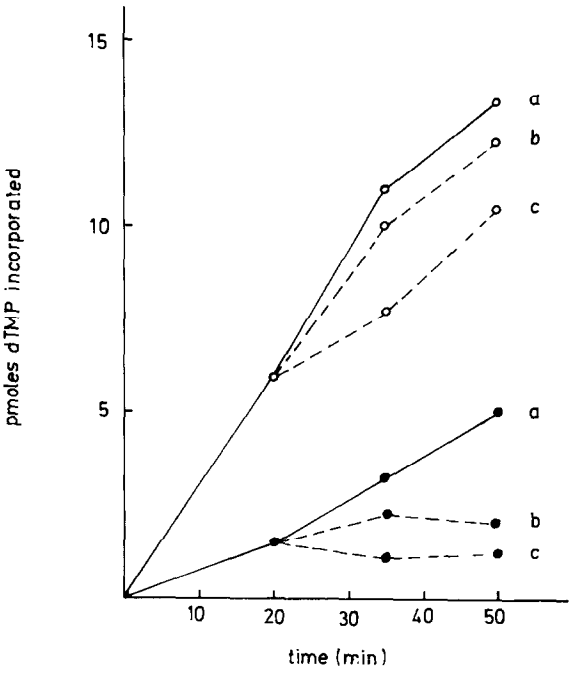

Fig.3. Effect of binding protein on DNA polymerase α activity at different concentrations of primer template. Heat-denatured calf thymus DNA, 25 μg, and binding protein, 100 μg, were incubated for 5 min at 37°C in 0.2 ml standard buffer. A control sample contained only DNA. Polymerisation was started by addition of 0.8 ml reaction mixture (spec.act. d[^{3}H]TTP: 600 cpm/pmol) containing 2 units DNA polymerase α. After 20 min at 37°C, 0.1 ml samples were removed to determine the incorporated radioactivity. The remaining mixtures were divided into three 0.2 ml samples: (a) the first sample remained undiluted; (b) the second sample was diluted 3-fold with 0.4 ml incubation mixture without DNA (but with nucleotides); (c) the third sample was diluted 10-fold with 1.8 ml incubation mixture without DNA (but with nucleotides). All samples were further incubated at 37°C and at the indicated times 0.1 ml (a), 0.3 ml (b) and 1.0 ml (c), respectively, were removed to determine the incorporated radioactivity: (○) acid-precipitable radioactivity in the presence of the binding protein; (●) acid-precipitable radioactivity in the absence of binding protein.

These negative results do not, of course, exclude a direct interaction of both components in the absence of DNA (particularly since the α-polymerase used in these studies is not completely purified from contaminating proteins). It is more likely, however, that the formation of a ternary complex which includes DNA, binding protein and α-polymerase is the essential step preceding the polymerisation reaction. This possibility is supported by the observation that the fraction of DNA bound α-polymerase is increased in the presence of binding protein, even at elevated salt concentrations (fig.1).

These ternary complexes are much more resistant to dilution than systems consisting of α-polymerase and DNA only (fig.3). An obvious interpretation of this result has been mentioned above: in the absence of binding protein, α-polymerase frequently leaves its template during polymerization and reassociates again [9] while in the presence of binding protein a more stable association of α-polymerase and DNA occurs. This interpretation predicts that polydeoxynucleotides synthesized in the presence of binding protein should be longer than those polymerized in the absence of this protein. This prediction is currently under investigation.

Acknowledgement

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